

# (12) UK Patent Application (19) GB (11) 2 304 107 (13) A

(43) Date of A Publication 12.03.1997

(21) Application No 9616282.1

(22) Date of Filing 01.08.1996

(30) Priority Data

(31) 9525992  
9516244

(32) 20.12.1995  
08.08.1995

(33) GB

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(51) INT CL<sup>6</sup>

C12N 9/08, C07D 231/08, C11D 3/386, C12N 9/96

(52) UK CL (Edition O)

C3H HK4  
C2C CAA CSF C1401 C215 C22Y C220 C25Y C250 C252  
C30Y C346 C352 C385 C51X C510 C536 C80Y C800  
C808  
C5D DHC D107 D110 D111 D117 D120 D126 D127 D142  
D147 D149 D153 D166 D173 D180 D182  
U1S S1333 S1400 S1427 S1527 S1597

(56) Documents Cited

WO 94/29510 A1 WO 94/29425 A2 WO 94/12621 A1

(58) Field of Search

UK CL (Edition O) C3H HK1 HK4, C5D DEX DHC  
INT CL<sup>6</sup> C11D 3/386, C12N 9/00 9/02 9/08 9/96  
ONLINE: WPL/CLAIMS

(54) Enzyme activity enhancement by addition of an (hetero)aromatic compound

(57) A process for the enhancement of the activity of an enzyme, especially in a detergent composition, comprises the addition thereto, as mediator for such enhancement, a compound of the formula:

[A].(X)x.(Y)y.(Z)z

wherein: A is an (hetero)aromatic radical;

X is -N=S or -N=O;

Y is -SH or -OH;

Z is a water-solubilising group;

x is 1 or 2; y is 1, 2 or 3;

z is 0, 1, 2, 3 or 4;

or a tautomer, or transition metal complex, thereof.

The enhanced active enzyme may be used:

(i) in the degradative bleaching of pulp for paper production;

(ii) in the degradative bleaching of stains on textiles during washing;

(iii) in the modification, breakdown or bleaching of lignin, especially during the production of paper

pulp;

(iv) in the degradation of organic material (especially dyestuffs) in waste water;

(v) in the degradative bleaching of migrating dyes in a washing process, so that the transfer of such dyes is inhibited.

The enzyme is preferably an oxidoreductase, especially an oxidase, peroxidase (particularly lignin peroxidase or manganese peroxidase) or laccase.

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### Enzyme Activity Enhancement

The present invention relates to a method of enhancing the activity of an enzyme with an enhancing agent for the enzyme and to the use of the enzyme of enhanced activity in a degradation process.

The use of a peroxidase enzyme in a process for the degradation, e.g. by bleaching, of a wide variety of substrates is already known.

For example, the use is known of a peroxidase enzyme in the bleaching of pulp for paper production, in the bleaching of textiles during washing, in the inhibition of dye transfer during washing and in the modification, breakdown or bleaching of lignin, especially during the production of paper pulp. In these known processes, the peroxidase enzyme is conventionally used together with hydrogen peroxide or a compound capable of producing hydrogen peroxide *in situ*.

Bourbonnais *et al.*, in FEBS letters (1990) 267, No.1, 99-102, have noted that, in the presence of certain compounds, termed mediators, such as the reactive dye Remazole Blue® or 2,2'-azinobis(3-ethylbenzthiazoline-6-sulfonate) (ABTS), laccase enzymes (polyphenol oxidases) can oxidize not only phenolic but also non-phenolic lignin subunits.

Although ABTS exhibits an excellent performance as a mediator for the bleaching of undesired dyestuffs, e.g. in the inhibition of dye transfer during washing, it is itself a dyestuff and suffers from the disadvantage that, after effecting its bleaching action, residual amounts of ABTS always remain in the treatment system.

In WO 94/12621, it has been proposed to use, as enhancers of the activity of peroxidases, organic chemical compounds consisting of at least two aromatic rings, at least one of which rings is substituted by one or more nitrogen, oxygen or sulfur atoms, the rings being optionally fused.

As merely one of many preferred sub-groups of such aromatic compounds, WO 94/12621 proposes naphthalene compounds which are optionally substituted by a wide variety of

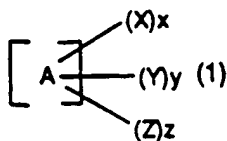
groups in all possible positions on the naphthalene nucleus. The disclosed substituent groups, however, do not include the nitroso group.

Moreover, in WO 94/29510, there is described a process for modifying, breaking down or bleaching lignin using oxidation catalysts and suitable oxidizing agents. The catalysts are used in combination with an aliphatic, cycloaliphatic, heterocyclic or aromatic compound containing NO-, NOH- or H-N(R)-OH groups (in which R is not defined). Within this wide scope of compounds, N-hydroxy heterocyclic compounds such as hydroxybenzotriazole appear to be preferred. No aromatic compounds containing a nitroso group are specifically mentioned.

1-Hydroxybenzotriazole is indeed an excellent mediator. It is probably not sufficiently economic, however, for use in bleaching applications. Moreover, during enzyme reaction involving 1-hydroxybenzotriazole, there is obtained the by-product benzotriazole, which has been found to be mutagenic.

Surprisingly, it has now been found that using, as a mediator in the degradation of a substrate in the presence of an oxidoreductase enzyme, a (hetero)aromatic compound containing both a hydroxy and a nitroso group, and/or the analogous sulfur compounds, excellent levels of substrate degradation are obtained.

Accordingly, the present invention provides, as a first aspect, a method for enhancing the activity of an enzyme, comprising the use of the enzyme in combination with a mediator compound having the formula:



in which A is a (hetero)aromatic residue; X is an -N=S or, preferably, an -N=O group; Y is an -SH or, preferably, an -OH group; Z is a water-solubilising group, preferably a group -SO<sub>2</sub>NH<sub>2</sub>, -CO<sub>2</sub>M or -SO<sub>3</sub>M in which M is hydrogen or a cation; x is 1 or 2, preferably 1; y is 1, 2 or 3, preferably 1; and z is 0, 1, 2, 3 or 4, preferably 0, 1 or 2.

The mediator compound of formula (1) may be present in any of its possible tautomeric forms and/or transition metal complex forms. When present as a transition metal complex, the mediator compound of formula (1) is preferably in the form of an iron, manganese or cobalt metal complex. Moreover, in addition to the essential substituents X and Y, the residue A may also contain one or more further substituents such as C<sub>1</sub>-C<sub>4</sub>alkyl, C<sub>1</sub>-C<sub>4</sub>alkoxy, halogen and nitro groups.

The enzyme component, the activity of which is to be enhanced according to the present invention, may be any oxidoreductase or any fraction or synthetic mimic of such an enzyme. By enzyme mimics are meant compounds which simulate the prosthetic group of an oxidoreductase. Examples of oxidoreductases include oxidases, peroxidases, lignin peroxidases, manganese peroxidases and, in particular, laccases and laccase related enzymes.

The peroxidase enzyme may be any of those comprised by the enzyme classification EC 1.11.1.7, or any peroxidase activity-exhibiting fragment derived from such enzyme. Also of interest are synthetic or semi-synthetic derivatives of such enzymes, such as those comprising porphyrin ring systems or microperoxidases which are described, e.g., in US-4077768, EP-537381, WO 91/05858 or WO 92/16634. Other relevant enzymes are peroxidase-active fragments derived from cytochromes, hemoglobin or peroxidase enzymes, or synthetic or semi-synthetic derivatives thereof such as iron porphins, iron porphyrins, iron phthalocyanine and derivatives thereof. Such enzymes may be derived from animal, vegetable or microbial sources.

The laccase or related laccase enzyme may be any of those comprised by the enzyme classification EC 1.10.3.2, any catechol oxidase enzyme comprised by the enzyme classification EC 1.10.3.1, any bilirubin oxidase enzyme comprised by the enzyme classification EC 1.3.3.5 or any monophenol monooxygenase enzyme comprised by the enzyme classification EC 1.14.99.1.

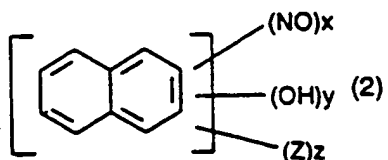
The laccase or related laccase enzyme may also be one which can be produced by cultivation of a host cell transformed with a recombinant DNA vector carrying a DNA sequence encoding the laccase and carrying DNA sequences encoding functions allowing

the expression of the DNA sequence encoding the laccase. The cultivation may be conducted in a culture medium under conditions which permit the expression of the laccase enzyme. Finally, the laccase may be recovered from the culture medium.

The laccases are preferably produced from white rotting fungi, especially *Phanerochaete chrysosporium* and *Coriolus (Trametes) versicolor*.

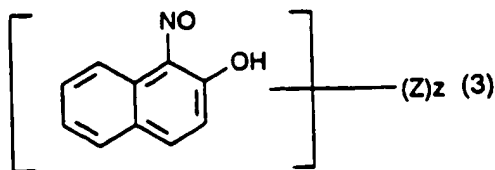
In the mediator compounds of formula (1), A may be a purely aromatic residue, preferably a phenyl residue or, especially, a naphthyl residue, or it may be a heteroaromatic residue, that is an aromatic residue containing one or more heteroatoms, preferably nitrogen, oxygen or sulfur atoms, such a pyrimidine or coumarin residue, or an aromatic residue substituted with a heterocyclic residue. Both aromatic and heteroaromatic residues A include mono- and polycyclic residues. In the case of the latter, the individual rings may be fused together, or linked together via a bridging member such as a direct bond,  $-(CH_2)_n-$ ,  $-(CH=CH)_n-$ ,  $-N=N-$ ,  $-O-$ ,  $-CO-$ ,  $-NH-$ ,  $-N=N-$ ,  $-S-$  or  $-SO_2-$  in which n is an integer from 1 to 6.

Examples of preferred purely aromatic mediator compounds of formula (1) are those having the formula:

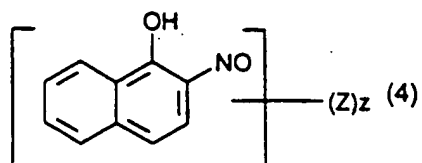


in which Z, x, y and z have their previous significance.

Preferred purely aromatic compounds of formula (2) are those having the formula:

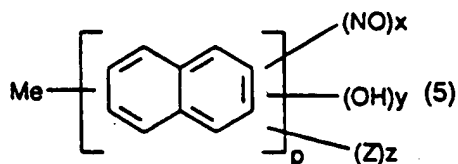


or the formula:



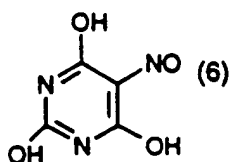
in which Z and z have their previous significance.

Further examples of preferred purely aromatic mediator compounds of formula (1) are those having the formula:

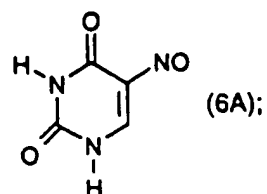


in which Z, x, y and z have their previous significance, Me is a transition metal and p is the valency of the transition metal Me.

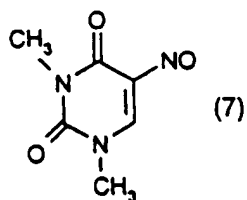
Examples of preferred heteroaromatic mediator compounds of formula (1) are pyrimidine derivatives having the formula:



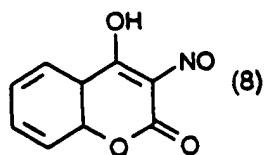
(the commercially available compound violuric acid), which can also exist in other tautomeric forms such as that having the formula:



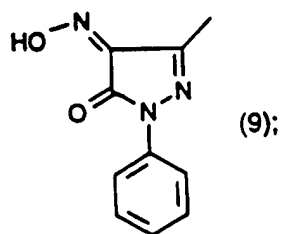
or having the formula:



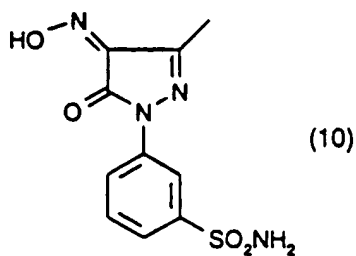
(1,3-dimethyl-5-nitrosobarbituric acid; Beilstein Ell, vol. 24, 307); and coumarin derivatives such as that having the formula:



(4-hydroxy-3-nitrosocoumarin; Beilstein E III/IV, vol. 17, 6737); as well as compounds having the formula:



(US-A-2 646 409, Example 19); or having the formula:

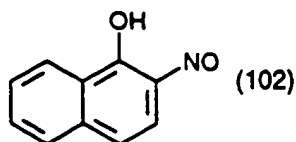
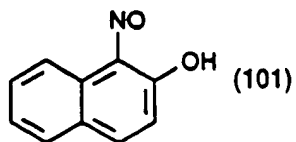


(having m.pt 230°C.(decomp.) and the elemental analysis (calculated for  $C_{10}H_{10}N_4SO_3$ ) C=42.85; H=3.57; N=19.85; S=11.36; O=22.67; found C=42.7; H=3.5; N=19.8; S=11.3; O=22.7 and produced analogously to the procedure described in US-A-2 646 409, Example 19).

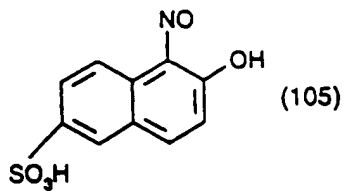
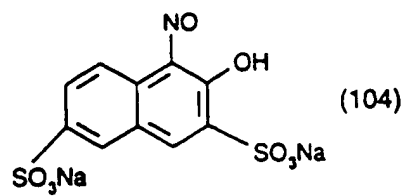
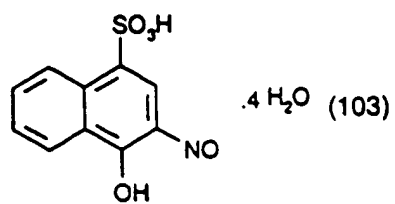
In the compounds of formula (1), (2), (3) or (4), when M is a cation, it is preferably an alkali metal cation, especially a sodium cation, or a cation formed from an amine.

When, in the compounds of formula (1), (2), (3) or (4), Z is a water-solubilising group, then it will be understood that the compounds of formula (1), (2), (3) or (4) may be used in the form of any desired hydrate.

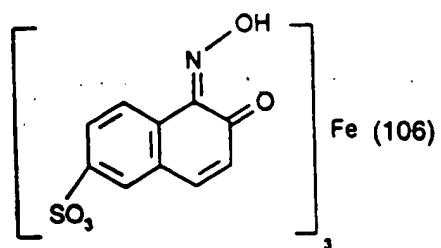
Particularly preferred purely aromatic mediator compounds of formula (1) include those having one of the formulae:



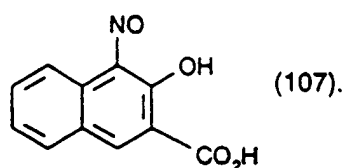
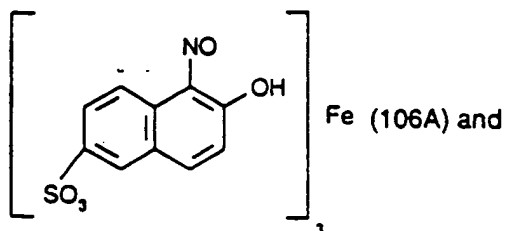




(Beilstein E II, vol. 11, page 150).



(C.I.10020; Acid Green B; Naphthol Green B); which may be in the tautomeric form having the formula:



(Berichte Deutschen Chem. Ges. 42, page 4266).

The mediator compounds are mostly known compounds and can be obtained commercially or by methods which are well known. The compound of formula (10), however, is a new compound and, as such, forms a second aspect of the present invention.

The present invention provides, as a third aspect, a process for the degradation of a substrate comprising contacting the substrate with a bleach liquor comprising an oxidizing agent, an enzyme and a mediator for the enzyme, according to the first aspect of the present invention.

If a peroxidase enzyme is used, the oxidizing agent used in the degradation process according to the present invention is preferably hydrogen peroxide or a compound capable of producing hydrogen peroxide *in situ*, such as a perborate, a percarbonate, a peroxy-carboxylic acid or a salt thereof.

On the other hand, if a laccase or laccase-related enzyme is used, the oxidizing agent used in the degradation process according to the present invention is preferably oxygen, conveniently supplied as air or pure oxygen, optionally with the application of pressure.

In addition to the above-mentioned enzymes, the degradation process of the invention may also include the addition of hemicellulases, cellulases, pectinases, amylases and lipases to the reaction mixture. Mixtures of two or more enzymes may also be used and, under certain circumstances, synergistic effects may be observed with the use of such mixtures of enzymes.

The oxidizing agent is preferably added at the start of the degradation process and preferably in an amount of from 0.0001 to 5.0% by weight, more preferably from 0.01 to 1.0% by weight, based on the total weight of the substrate to be degraded.

The degradation process according to the present invention is preferably applied to the degradative bleaching of pulp for paper production; in the degradative bleaching of stains on a) textiles during washing thereof with laundry detergents or on b) dishes during washing thereof with dishwashing detergents; in the modification, breakdown or bleaching of lignin, especially during the production of paper pulp; in the degradation of organic material such as a dyestuff in waste water; and in the degradative bleaching of migrating dyes in a washing process, so that inhibition of transfer of such dyes during washing is effected.

When the process of the present invention is applied to degradative bleaching of pulp, the process is generally conveniently operated at a temperature in the range of from 25-80°C., preferably at a temperature in the range of from 40-60°C. The process is preferably conducted in the presence of air or oxygen and at a pressure ranging from normal pressure up to 10 bar excess pressure. The consistency of the process reaction mixture preferably ranges from 0.5-40%.

The process of the present invention may be applied to the production of pulps in general. Thus, the process may be used for the delignification (bleaching) of sulfate-, sulfite-, organosolve- and wood pulp.

The present invention further provides a method for the degradative bleaching of stains on a) textiles during washing thereof with laundry detergents or on b) dishes during washing thereof with dishwashing detergents, comprising washing the textiles or dishes with a

laundry or dishwashing detergent which contains an enzyme having enhanced activity by the addition of a mediator compound of formula (1) according to the present invention.

The present invention also provides a method for inhibiting the re-absorption of migrating dyes in the wash liquor, comprising introducing into a wash liquor containing a peroxide detergent, from 0.001 to 150 mg, per litre of wash liquor, of an enzyme having enhanced activity by the addition of a mediator compound of formula (1) according to the present invention.

When the method of the present invention is applied to the degradative bleaching of stains on textiles during washing thereof with laundry detergents, or for inhibiting the re-absorption of migrating dyes in the wash liquor, comprising introducing into a wash liquor containing a peroxide detergent, the method is generally conveniently operated at a temperature in the range of from 15-80°C., preferably at a temperature in the range of from 25-50°C.

The present invention further provides a detergent composition comprising:

- i) 5-90%, preferably 5-70% of A) an anionic surfactant and/or B) a nonionic surfactant;
- ii) 5-70%, preferably 5-50%, especially 5-40% of C) a builder;
- iii) 0.01-30%, preferably 1-12% of D) a peroxide; and
- iv) 0.001-2%, preferably 0.02-2% of E) an enzyme having enhanced activity by the addition of a mediator compound of formula (1) according to the present invention, each by weight, based on the total weight of the detergent.

Each of components A) to E) may be a single compound or a mixture of compounds.

The detergent may be formulated as a solid; or as a non-aqueous liquid detergent, containing not more than 5, preferably 0-1 wt.% of water, and based on a suspension of a builder in a non-ionic surfactant, as described, e.g., in GB-A-2158454.

Preferably, the detergent is in powder or granulate form.

Such powder or granulate forms may be produced by firstly forming a base powder by spray-drying an aqueous slurry containing all the said components, apart from the components D) and E); then adding the components D) and E) by dry-blending them into

the base powder. In a further process, the component E) may be added to an aqueous slurry containing components A), B) and C), followed by spray-drying the slurry prior to dry-blending component D) into the mixture. In a still further process, component B) is not present, or is only partly present in an aqueous slurry containing components A) and C); component E) is incorporated into component B), which is then added to the spray-dried base powder; and finally component D) is dry-blended into the mixture.

The anionic surfactant component A) may be, e.g., a sulphate, sulphonate or carboxylate surfactant, or a mixture of these.

Preferred sulphates are alkyl sulphates having 12-22 carbon atoms in the alkyl radical, optionally in combination with alkyl ethoxy sulphates having 10-20 carbon atoms in the alkyl radical.

Preferred sulphonates include alkyl benzene sulphonates having 9-15 carbon atoms in the alkyl radical.

In each case, the cation is preferably an alkali metal, especially sodium.

Preferred carboxylates are alkali metal sarcosinates of formula  $R-CO(R')CH_2COOM^1$  in which R is alkyl or alkenyl having 9-17 carbon atoms in the alkyl or alkenyl radical, R' is  $C_1-C_4$ alkyl and M<sup>1</sup> is alkali metal.

The nonionic surfactant component B) may be, e.g., a condensate of ethylene oxide with a  $C_6-C_{15}$  primary alcohol having 3-8 moles of ethylene oxide per mole.

The builder component C) may be an alkali metal phosphate, especially a tripolyphosphate; a carbonate or bicarbonate, especially the sodium salts thereof; a silicate; an aluminosilicate; a polycarboxylate; a polycarboxylic acid; an organic phosphonate; or an aminoalkylene poly (alkylene phosphonate); or a mixture of these.

Preferred silicates are crystalline layered sodium silicates of the formula  $\text{NaHSi}_m\text{O}_{2m+1}$  or  $\text{Na}_2\text{Si}_m\text{O}_{2m+1} \cdot p\text{H}_2\text{O}$  in which  $m$  is a number from 1.9 to 4 and  $p$  is 0 to 20.

Preferred aluminosilicates are the commercially-available synthetic materials designated as Zeolites A, B, X, and HS, or mixtures of these. Zeolite A is preferred.

Preferred polycarboxylates include hydroxypolycarboxylates, in particular citrates, polyacrylates and their copolymers with maleic anhydride.

Preferred polycarboxylic acids include nitrilotriacetic acid and ethylene diamine tetra-acetic acid.

Preferred organic phosphonates or aminoalkylene poly (alkylene phosphonates) are alkali metal ethane 1-hydroxy diphosphonates, nitrilo trimethylene phosphonates, ethylene diamine tetra methylene phosphonates and diethylene triamine penta methylene phosphonates.

The peroxide component D) may be any organic or inorganic peroxide compound, described in the literature or available on the market, which bleaches textiles at conventional washing temperatures, e.g. temperatures in the range of from 10°C. to 90°C. In particular, the organic peroxides are, for example, monoperoxides or polyperoxides having alkyl chains of at least 3, preferably 6 to 20, carbon atoms; in particular diperoxydicarboxylates having 6 to 12 C atoms, such as diperoxyperazates, diperoxypersebacates, diperoxyphthalates and/or diperoxydodecanedioates, especially their corresponding free acids, are of interest. It is preferred, however, to employ very active inorganic peroxides, such as persulphate, perborate and/or percarbonate. It is, of course, also possible to employ mixtures of organic and/or inorganic peroxides. Peroxides can have different crystalline forms and/or different degrees of hydration. They may be used in admixture with other organic or inorganic salts, thereby improving their stability to storage.

The addition of the peroxides to the detergent is effected, in particular, by mixing the components, for example by means of screw-metering systems and/or fluidized bed mixers.

The detergents may contain, in addition to the combination according to the invention, one or more of fluorescent whitening agents, such as a bis-triazinylamino-stilbene-disulphonic acid, a bis-triazolyl-stilbene-disulphonic acid, a bis-styryl-biphenyl, a bis-benzofuranyl-biphenyl, a bis-benzoxalyl derivative, a bis-benzimidazolyl derivative, a coumarine derivative or a pyrazoline derivative; soil suspending agents, for example sodium carboxymethylcellulose; salts for adjusting the pH, for example alkali or alkaline earth metal silicates; foam regulators, for example soap; salts for adjusting the spray drying and granulating properties, for example sodium sulphate; perfumes; and also, if appropriate, antistatic and softening agents; such as smectite clays; enzymes, such as amylases; photobleaching agents; pigments; and/or shading agents. These constituents should, of course, be stable to the bleaching system employed.

A particularly preferred detergent co-additive is a polymer known to be useful in preventing the transfer of labile dyes between fabrics during the washing cycle. Preferred examples of such polymers are polyvinyl pyrrolidones, optionally modified by the inclusion of an anionic or cationic substituent, especially those having a molecular weight in the range from 5000 to 60,000, in particular from 10,00 to 50,000. Preferably, such polymer is used in an amount ranging from 0.05 to 5%, preferably 0.2-1.7% by weight, based on the weight of the detergent.

The following Examples further illustrate the present invention.

#### Examples 1 to 3

The oxidation of C.I. Direct Blue 1 is monitored spectroscopically at 610 nm (the absorption peak of the dye) in a spectrophotometer.

The reagents are added, using a pipette, in the following sequence, to a cuvette having a layer thickness of 1 cm:

acetate buffer pH 5

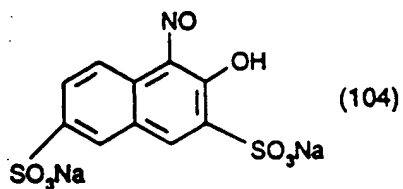
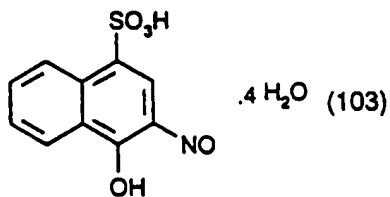
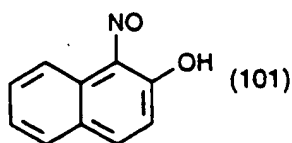
C.I.Direct Blue 1

H<sub>2</sub>O

mediator under test and

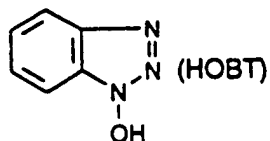
laccase enzyme (polyphenol oxidase produced from *Trametes versicolor*).

The test mediators used according to the present invention are those having the following formulae:



For the purpose of comparison, an experiment is conducted using, as mediator, the known mediator compound N-hydroxybenzotriazole having the formula:





Directly after the addition of the enzyme, the mixture is stirred with the tip of the pipette for about 15 seconds. 20 seconds after the addition of the enzyme, the spectroscopic measurement at 610 nm is commenced and the spectrum is monitored for 8 minutes. The temperature of the reagents and the cuvette is set at 25°C. Each experiment is conducted at pH 5. The volume of liquid in the cuvette is adjusted to 3 ml for each experiment by adding the necessary amount of water.

In the following Table 1, there are shown, for each reagent, the concentration of the reagent in the basic solution, the amount of the sample of reagent removed and the concentration of the reagent in the final solution.

**Table 1**

Reagent	Concentration of basic solution	Amount of sample taken	Final concentration in cuvette
acetate buffer pH 5	2.86 mole/l	25 $\mu$ l	$20 \cdot 10^{-3}$ mole/l
Direct Blue 1	$7.54 \cdot 10^{-4}$ mole/l	100 $\mu$ l	$2.51 \cdot 10^{-5}$ mole/l
mediator: Compound 101 Compound 103 Compound 104 HOBT	$1.42 \cdot 10^{-3}$ mole/l	21 $\mu$ l	$10 \cdot 10^{-6}$ mole/l
laccase	6.0 U/ml	50 $\mu$ l	0.1 U/ml

The concentration unit U ( $\mu$ mol/min) given for the laccase is defined in Bourbonnais *et al.*, in FEBS letters (1990) 267, No.1, 99-102 (supra).

The results of the dye oxidation tests, showing the optical density (OD) measurements at 0 and at 8 minutes, and the rate of increase (initial gradient) of the respective OD curves, are set out in the following Table 2.

Table 2

Mediator	OD <sub>0 min</sub>	OD <sub>8 min</sub>	Initial Gradient x 1000
none (laccase alone)	0.90	0.67	31.73
HOBt	0.90	0.56	54.18
Compound 101	0.83	0.40	231.17
Compound 103	0.90	0.36	79.72
Compound 104	0.89	0.33	108.98

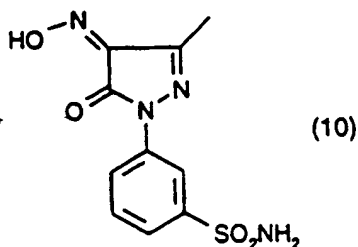
The rate of dye oxidation obtained with the mediators used according to the invention is clearly greatly superior to that in the control experiment or using the known mediator HOBt.

Similar results are obtained when the compounds of formula (101), (103) and (104) are replaced by those of formula (105), (106), (107), (6), (7), (8), (9) or (10).

Example 4

1.0 ml of 1M H<sub>2</sub>SO<sub>4</sub> is added to of moist softwood pulp (dry softwood pulp content 10.0 g ) in order to maintain the pulp at an acidic pH.

0.05 g of a mediator compound of formula:



is dissolved in 20 mls of water. This mediator solution, together with 4 mls of a 0.5% by weight aqueous solution of EDTA and - mls of water are then added to the pulp in order to provide, in the mixture, a concentration of 5 mg of the mediator compound of formula (10) per g of pulp and a concentration of 2 mg of EDTA per g of pulp. Over a period of 60 seconds, the resulting mixture is thoroughly homogenized. To the homogenized mixture there are added Versicolor laccase (EC number 1.10.3.2), 1.9 mls of an aqueous 2.6M sodium acetate buffer solution (pH 5), 0.25 ml of a 20% by weight aqueous solution of  $\text{MgSO}_4$ , to give a concentration of 3.5 U of laccase per g of pulp, a concentration of 0.5 mg of  $\text{MgSO}_4$  per g of pulp and a concentration of 50 mM of sodium acetate in the resulting mixture. The mixture so obtained is kneaded for 60 seconds and the pH thereof is adjusted to 5, if necessary, by the addition of further 1M  $\text{H}_2\text{SO}_4$ . The total weight of the mixture is then adjusted to 100 g by the addition of further water.

The kneaded mixture is placed in a steel container from which the air is then evacuated using a water jet pump. The container is then charged with oxygen to a pressure of 3 bar. The container is then heated at 50°C. for 225 minutes and subsequently the oxygen is removed using a water jet pump.

The reaction mixture is washed with 1 litre of warm (50°C.) water in a Buchner funnel; then extracted with a warm (70°C.) aqueous NaOH solution, using 2 g of NaOH per 100 g of pulp; and finally re-washed with 1 litre of warm (50°C.) water in a Buchner funnel. The total weight of the alkaline mixture (including 10 g of pulp) is then adjusted to 100 g by the addition of further water.

The Kappa number of the washed material is then determined according to the test method SCAN-C 1:77 of the Scandinavian Pulp and Paper Board. Compared with the initial Kappa number, a Kappa number reduction of 26% is obtained.

#### Example 5

Using a beaker laboratory dyeing apparatus having 50 mls containers, respective wash liquids are made up containing, in 25 mls of water, a) a borax buffer (pH 9) or a commercial detergent; b) a peroxidase from horse radish (Fluka No. 77333) dissolved in a phosphate buffer (pH 6); and c) the compound of formula (101). The wash liquor is pre-heated to 40°C. Test cotton swatches are immersed in the respective wash liquids and then 5 mls of an aqueous solution, also pre-heated to 40°C., of Cibacron Marine C-B dye and hydrogen peroxide are added.

The reagent concentrations are shown in the following Table 3.

Table 3

Reagent	Final concentration in wash liquor	
	buffer system	detergent system
borax buffer	20 mM	-
commercial detergent	-	4.4 g/l
H <sub>2</sub> O <sub>2</sub>	200 µM	200 µM
compound (101) (stock solution 20:80 ethanol/water)	90 µM	90 µM
peroxidase, 727 U/mg	0.56 U/ml	0.56 U/ml
Cibacron Marine C-B	20 µM	8 µM
cotton swatches	2.8 g	3.75 g

The total volume in the wash container is 30 mls.

1U of Fluka No. 77333 corresponds to the amount of enzyme which oxidizes 1 $\mu$ mol of ABTS per minute at pH 6 and at 25°C.

During the washing process, the temperature is held at 40°C. for 30 minutes. The swatches are then removed from the containers, rinsed with water, dried and ironed. The brightness values (Y) of the washed samples, as well as those of control samples, are then determined spectrophotometrically. the results are set out in the following Table 4.

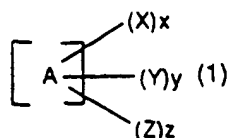
Table 4

Example	wash conditions	Y	
		buffer system	detergent system
-	control: cotton untreated	92.5	92.5
-	control: no enzyme or compound (101)	72.8	82.0
-	control: enzyme but no compound (101)	74.8	82.3
5	complete system	85.4	90.0

The results in Table 4 demonstrate that the method of the present invention is very effective in inhibiting the transfer of the dye on to the cotton and thus maintains, to a great extent, the original brightness of the washed cotton. The results obtained compare favourably with those resulting from the use of a commercial detergent.

### Claims

1. A method for enhancing the activity of an enzyme, comprising adding to the enzyme, as a mediator for enhancing the activity of the enzyme, a compound having the formula:



in which A is a (hetero)aromatic residue; X is an -N=S or an -N=O group; Y is an -SH or an -OH group; Z is a water-solubilising group; x is 1 or 2; y is 1, 2 or 3; and z is 0, 1, 2, 3 or 4; or a tautomer or transition metal complex of a compound of formula (1).

2. A method according to claim 1 in which X is an -N=O group.

3. A method according to claim 1 or 2 in which Y is an -OH group.

4. A method according to any of the preceding claims in which Z is a group -SO<sub>2</sub>NH<sub>2</sub>, -CO<sub>2</sub>M or -SO<sub>3</sub>M in which M is hydrogen or a cation.

5. A method according to any of the preceding claims in which x is 1.

6. A method according to any of the preceding claims in which y is 1.

7. A method according to any of the preceding claims in which z is 0, 1 or 2.

8. A method according to any of the preceding claims in which the enzyme component is an oxidoreductase or any fraction or synthetic mimic of such an enzyme.

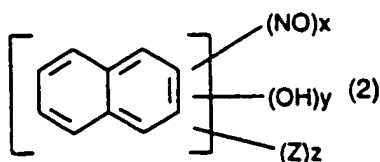
9. A method according to any of the preceding claims in which the oxidoreductase is an oxidase, peroxidase, lignin peroxidase, manganese peroxidase or laccase.

10. A method according to claim 9 in which the oxidoreductase is a laccase or laccase related enzyme.

11. A method according to any of the preceding claims in which a compound of formula (1) is used in which A is an aromatic residue.

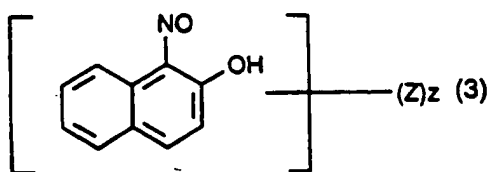
12. A method according to claim 11 in which A is a naphthyl residue.

13. A method according to claim 12 in which the compound of formula (1) has the formula:

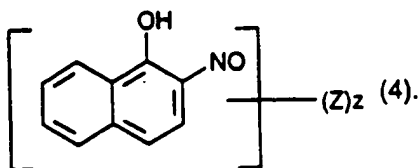


in which Z, x, y and z are as defined in claim 1.

14. A method according to claim 13 in which the compound of formula (2) has the formula:

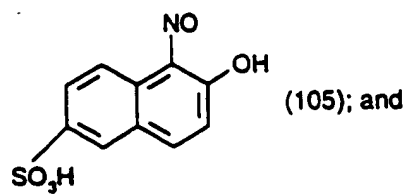
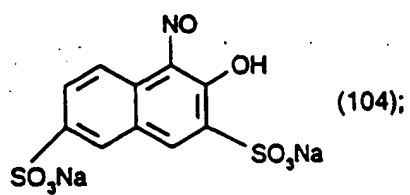
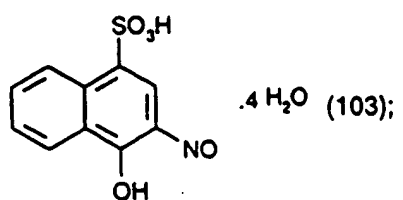
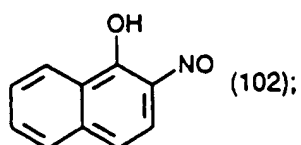
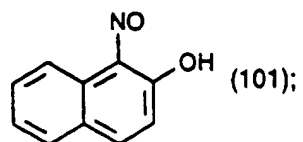


or the formula:

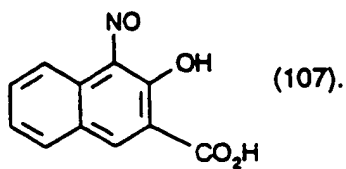


in which Z and z are as defined in claim 1.

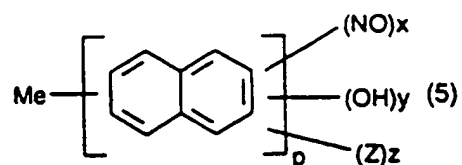
15. A method according to claim 14 in which the compound of formula (1) has one of the formulae:





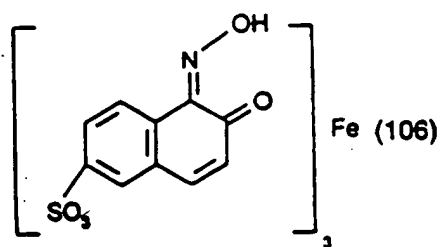


16. A method according to claim 12 in which the compound of formula (1) has the formula:

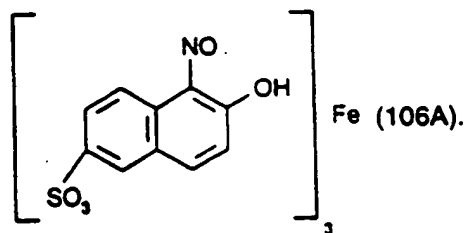


in which Z, x, y and z are as defined in claim 1, Me is a transition metal and p is the valency of the transition metal Me.

17. A method according to claim 16 in which the compound of formula (5) has the formula:

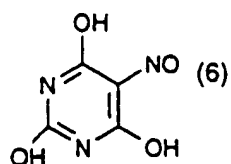


or the tautomeric form having the formula:

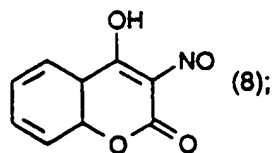
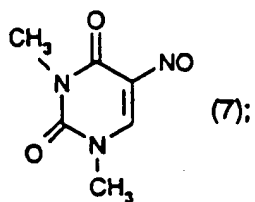
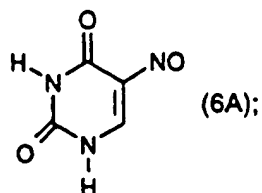


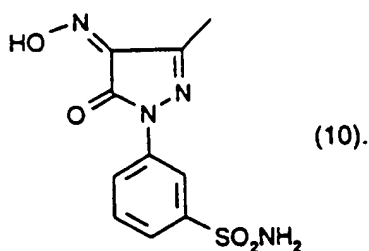
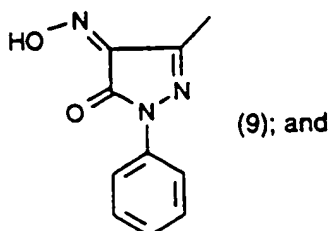
18. A method according to any of claims 1 to 10 in which a compound of formula (1) is used in which A is an heteroaromatic residue.

19. A method according to claim 18 in which the compound of formula (1) has one of the formulae:



or the tautomeric form having the formula:





20. A method according to any of the preceding claims in which, in the compounds of formula (1), (2), (3) or (4), when M is a cation, it is an alkali metal cation or a cation formed from an amine.

21. A method according to any of the preceding claims in which when, in the compounds of formula (1), (2), (3) or (4), Z is a water-solubilising group, then the compounds of formula (1), (2), (3) or (4) are used in the form of any desired hydrate.

22. A process for the degradation of a substrate comprising contacting the substrate with a bleach liquor comprising an oxidizing agent, an enzyme and a mediator compound of formula (101) for the enzyme as defined in any of claims 1 to 21.

23. A process according to claim 22 in which the oxidizing agent is added at the start of the process in an amount of from 0.0001 to 5.0% by weight, based on the total weight of the substrate.

24. A process according to claim 23 in which the oxidizing agent is added in an amount of from 0.01 to 1.0% by weight, based on the total weight of the substrate.

25. A process according to any of claims 22 to 24 in which a peroxidase enzyme is used and the oxidizing agent is hydrogen peroxide or a compound capable of producing hydrogen peroxide *in situ*.

26. A process according to claim 25 in which the oxidizing agent is a perborate, a percarbonate, a peroxy-carboxylic acid or a salt thereof.

27. A process according to any of claims 22 to 24 in which a laccase enzyme is used and the oxidizing agent is oxygen and is optionally applied under pressure.

28. A process according to any of claims 22 to 27 in which the degradation process is applied to the degradative bleaching of pulp for paper production.

29. A process according to any of claims 22 to 27 in which the degradation process is applied to the degradative bleaching of stains on textiles during washing with laundry detergents or to the degradative bleaching of stains on dishes during washing of the dishes with dishwashing detergents.

30. A process according to any of claims 22 to 27 in which the degradation process is applied to the modification, breakdown or bleaching of lignin.

31. A process according to claim 30 in which the degradation process is applied to the production of paper pulp.

32. A process according to any of claims 22 to 27 in which the degradation process is applied to the degradation of organic material such as a dyestuff in waste water.

33. A process according to claim 32 in which the organic material is a dyestuff.

34. A process for inhibiting the re-absorption of migrating dyes in the wash liquor, comprising introducing into a wash liquor containing a peroxide detergent, from 0.001 to 150 mg, per litre of wash liquor, of an enzyme having enhanced activity by virtue of the addition of a mediator compound according to any of claims 1 to 21.

35. A detergent composition comprising:

- i) 5-90% of A) an anionic surfactant and/or B) a nonionic surfactant;
- ii) 5-70% of C) a builder;
- iii) 0.01-30% of D) a peroxide; and
- iv) 0.001-2% of E) an enzyme having enhanced activity by the addition of a mediator compound according to any of claims 1 to 21, each by weight, based on the total weight of the detergent.

36. A detergent composition according to claim 35 comprising:

- i) 5-70% of A) an anionic surfactant and/or B) a nonionic surfactant;
- ii) 5-50% of C) a builder;
- iii) 1-12% of D) a peroxide; and
- iv) 0.001-2% of E) an enzyme having enhanced activity by the addition of a mediator compound according to any of claims 1 to 21, each by weight, based on the total weight of the detergent.

37. A composition according to claim 36 comprising:

- i) 5-70% of A) an anionic surfactant and/or B) a nonionic surfactant;
- ii) 5-40% of C) a builder;
- iii) 1-12% of D) a peroxide; and
- iv) 0.02-2% of E) an enzyme having enhanced activity by the addition of a mediator compound according to any of claims 1 to 21, each by weight, based on the total weight of the detergent.

38. A detergent composition according to any of claims 35 to 37 comprising 0.5-5% by weight of a polymer useful in preventing the transfer of labile dyes between fabrics during a washing cycle.

39. A detergent composition according to claim 38 comprising 0.2-1.7% of the polymer.

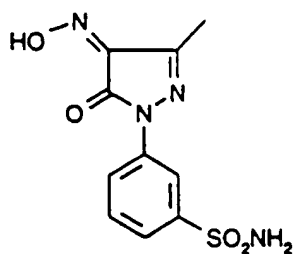
40. A detergent composition according to claim 38 or 39 in which the polymer is a polyvinylpyrrolidone optionally containing an anionic or cationic substituent.

41. A detergent composition according to any of claims 35 to 40 in which the detergent is in powder or granulate form.

42. A detergent composition according to any of claims 35 to 40 in which the detergent is in liquid form and contains 0-5% water.

43. A detergent composition according to claim 42 in which the detergent is in liquid form and contains 0-1% water.

44. A compound having the formula:

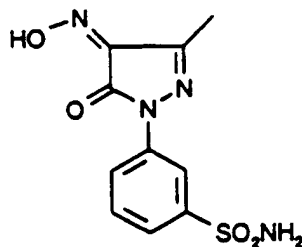


41. A detergent composition according to any of claims 35 to 40 in which the detergent is in powder or granulate form.

42. A detergent composition according to any of claims 35 to 40 in which the detergent is in liquid form and contains 0-5% water.

43. A detergent composition according to claim 42 in which the detergent is in liquid form and contains 0-1% water.

44. A compound having the formula:



45. A method according to claim 1 substantially as hereinbefore described with reference to any one of the foregoing Examples.



Application No: GB 9616282.1  
Claims searched: 1 to 43, 45

Examiner: Colin Sherrington  
Date of search: 2 October 1996

**Patents Act 1977**  
**Search Report under Section 17**

**Databases searched:**

UK Patent Office collections, including GB, EP, WO & US patent specifications, in:  
UK CI (Ed.O): C3H(HK1,HK4); C5D(DEX,DHC)  
Int CI (Ed.6): C11D 3/386; C12N 9/00,9/02,9/08,9/96;  
Other: ONLINE: WPI,CLAIMS

**Documents considered to be relevant:**

Category	Identity of document and relevant passage	Relevant to claims
A	WO 94/12621 A1 (NOVO NORDISK) -whole document	1
A	WO 94/29425 A2 (CALL, Hans-Peter) -whole document	1
A	WO 94/29510 A1 (CALL, Hans-Peter) -whole document	1

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